

CLAIMS

We claim:

1. A method for generating mRNA-cDNA hybrids, comprising the steps of:
 2. (a) providing: i) a solution comprising a nucleic acid template, ii) one or more primers sufficiently complementary to the sense conformation of said nucleic acid template, and iii) one or more promoter-linked primers sufficiently complementary to the antisense conformation of said nucleic acid template, and having an RNA promoter;
 3. (b) treating said nucleic acid template with said one or more primers under conditions such that a first cDNA strand is synthesized;
 4. (c) treating said first cDNA strand with said one or more promoter-linked primers under conditions such that a promoter-linked double-stranded nucleic acid is synthesized;
 5. (d) treating said promoter-linked double-stranded nucleic acid under conditions such that essentially amplified mRNA fragments are synthesized; and
 6. (e) treating said mRNA fragments with said one or more primers under conditions such that mRNA-cDNA hybrids are synthesized by reverse transcription of said amplified mRNA fragments with the extension of said one or more primers.
7. The method of Claim 1, further comprising the step of repeating steps b) through
8. e) for a sufficient number of cycles to obtain a desired amount of amplified
9. product.
10. The method of Claim 1, wherein said treating step in step b) comprises heating
11. said solution at a temperature above 90 °C to provide denatured nucleic acids.
12. The method of Claim 1, wherein said treating step in step c) comprises treating
13. said first cDNA strand with said one or more promoter-linked primers at a
14. temperature ranging from about 35 °C to about 75 °C.
15. The method of Claim 1, wherein said treating step in step c) comprises treating
16. said cDNA strand with one or more promoter-linked primers in the presence of a
17. polymerase.

- 1 6. The method of Claim 5, wherein said polymerase is selected from the group
2 consisting of DNA-dependent DNA polymerases, RNA-dependent DNA
3 polymerases, RNA polymerases, Taq-like DNA polymerase, TTh-like DNA
4 polymerase, *C. therm.* polymerase, viral replicases, and combinations thereof.
1 7. The method of Claim 6, wherein said viral replicases are selected from the group
2 consisting of avian myeloblastosis virus reverse transcriptase and Moloney
3 murine leukemia virus reverse transcriptase, and mutants thereof.
1 8. The method of Claim 7, wherein said avian myeloblastosis virus reverse
2 transcriptase does not have RNase activity.
1 9. The method of Claim 1, wherein said treating step in step d) comprises treating
2 said promoter-linked double-stranded nucleic acid with an enzyme having
3 transcriptase activity at about 37 °C.
1 10. The method of Claim 9, wherein said enzyme having transcriptase activity is
2 selected from the group consisting of RNA polymerases and viral replicases.
1 11. The method of Claim 10, wherein said RNA polymerases are selected from the
2 group consisting of T3 RNA polymerase, T7 RNA polymerase, SP6 RNA
3 polymerase, and M13 RNA polymerase.
1 12. The method as defined in Claim 1, wherein said treating step in step e) comprises
2 treating said mRNA fragments with said one or more primers at a temperature
3 ranging from about 35 °C to about 75 °C.
1 13. The method of Claim 1, wherein said one or more primers are complementary to
2 the 3'-ends of the sense conformation of said nucleic acid template.
1 14. The method of Claim 1, wherein said one or more primers comprises a poly(dT)₂₄
2 primer.
1 15. The method of Claim 1, wherein said one or more promoter-linked primers are
2 complementary to the 5'-ends of the antisense conformation of said nucleic acid
3 template.
1 16. The method of Claim 1, wherein said one or more promoter-linked primers
2 comprise oligo(dC)₁₀N-promoter primers.

17. The method of Claim 16, wherein said oligo(dC)₁₀N-promoter primers comprise a primer selected from the group consisting of oligo(dC)₁₀G-T7 primers, oligo(dC)₁₀A-T7 primers, oligo(dC)₁₀T-T7 primers, and combinations thereof.
 18. The method of Claim 1, wherein said promoter-linked double-stranded nucleic acid is a nucleic acid selected from the group consisting of promoter-linked double-stranded DNAs and promoter-linked double-stranded RNAs.
 19. The method of Claim 1, further comprising the step of incorporating one or more nucleotide analogs into the cDNA portion of said mRNA-cDNA hybrid to prevent degradation.
 20. The method of Claim 1, further comprising the step of contacting said mRNA-cDNA with a reagent for transfecting a eukaryotic cell for inhibiting the expression of a gene.
 21. The method of Claim 20, wherein said reagent is selected from the group consisting of chemical transfection reagents and liposomal transfection reagents.
 22. The method as defined in Claim 20, wherein said gene comprises a gene selected from the group consisting of pathogenic nucleic acids, viral genes, mutated genes, and oncogenes.
 23. A composition for inhibiting the expression of a targeted gene in a substrate, the composition comprising:
an mRNA-cDNA hybrid.
 24. The composition of claim 23, wherein the mRNA-cDNA hybrid is synthesized using the method of Claim 1.
 25. The composition of claim 23, wherein the composition is used to inhibit the expression of the targeted gene *in vivo*.
 26. The composition of claim 23, wherein the mRNA of said mRNA-cDNA hybrid is comprised of either part or all of the spliced mRNA transcript of the targeted gene.
 27. The composition of claim 23, wherein the mRNA of said mRNA-cDNA hybrid is comprised of part or all of the unspliced mRNA transcript of the targeted gene.
 28. The composition of claim 23, wherein the mRNA of said mRNA-cDNA hybrid is comprised of the combination of part or all of the unspliced and spliced mRNA transcript of the targeted gene.

- 1 29. The composition of claim 23, wherein the mRNA-cDNA hybrid is made by
2 complementarily combining the sense-oriented mRNA molecule of claim 26, 27
3 or 28 with its corresponding antisense-oriented cDNA molecule in a base-pairing
4 double-stranded form.
- 1 30. The composition of claim 23, wherein the substrate is a cell or an organism.
- 1 31. The composition of claim 23, further comprising a carrier molecule, which carrier
2 molecule is capable of being taken up by a cell.
- 3 32. A method for inhibiting the expression of a targeted gene in a substrate that
4 expresses the targeted gene, comprising the steps of:
5 a) providing a composition comprising an mRNA-cDNA hybrid capable of
6 inhibiting the expression of said targeted gene in said substrate; and
7 b) contacting said substrate with said composition under conditions such that
8 the expression of said gene in said substrate is inhibited.
9 33. The method of Claim 32, wherein said composition is the composition of claim
10 24.
- 11 34. The method of Claim 32, wherein said substrate expresses said targeted gene *in*
12 *vivo*.
- 13 35. The method of Claim 32, wherein said targeted gene comprises a gene selected
14 from the group consisting of pathogenic nucleic acids, viral genes, mutated genes,
15 and oncogenes.
- 16 36. The method of Claim 32, wherein said mRNA-cDNA hybrid inhibits β-catenin
17 expression.
- 18 37. The method of Claim 32, wherein said mRNA-cDNA hybrid inhibits bcl-2
19 expression.
- 20 38. The method of Claim 32, wherein said substrate is a prokaryote.
- 21 39. The method of Claim 38, wherein said prokaryote is a virus.
- 22 40. The method of Claim 38, wherein said prokaryote is a bacterial cell.
- 23 41. The method of Claim 32, wherein said substrate is a eukaryote or the cell of said
24 eukaryote.
- 25 42. The method of Claim 41, wherein said eukaryote is a vertebrate.
- 26 43. The method of Claim 41, wherein said eukaryote is a mouse.

- 1 44. The method Claim 41, wherein said eukaryote is a chimpanzee.
- 1 45. The method of Claim 41, wherein said eukaryote is a human being.

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